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Physiologisch-Chemisches Institut der Universität
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Tumors in rats caused by the damage resulting from the administration of malachite green to an ancestral generation

by

G. Werth

After the administration of toxic doses of malachite green rats exhibit the reactions symptomatic of asphyxiation, i.e. respiratory distress, cyanosis, finally spasms and death accompanied by bleeding from the nose and mouth. Mucosal hemorrhaging from the bladder, intestines and vagina also occurred occasionally. Examination of the ECG shows signs of oxygen deficiency and reveals the same symptoms typical of deaths occuring at high altitude or in the decompression chamber.

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Since exogenic oxygen deficiency can be excluded, an experiment was made to control the toxic effects of malachite green by administering an enzyme which is important in cell respiration, i.e. cytochrome c. This experiment proved that it is possible to prevent the animals from dying following malachite-green poisoning and, as traceable on the ECG, the effects of the noxa on cardiac action and function can be normalized.

These series of tests suggested that malachite green might poison the organism via the cytochrome system. For this reason, serial tests were carried out to obtain data: over a long period of time, the animals were systematically administered fixed quantities of malachite green; to be precise, we gave them 1/3 to 1/5 of the previously established lethal dose 50, i.e. 3 mg/100 mg of body weight. The rats were sacrificed at fixed intervals and a spectrophotometric analysis according to KEILIN and HARTREE was carried out to determine the cytochrome-c concentration in the heart muscle. It turned out that periodical administrations of malachite green made it possible to reduce the cytochrome-c concentration in the organism. The results obtained for these test animals and for our normal animals differed by up to 20% and more. Analogous to the observation that cytochrome-c is capable of preventing the malachite green damage registered on the ECG, it was also found that the simultaneous administration of malachite green and cytochrome-c did not lead to a reduction of the cytochrome-c concentration in the heart muscle. (Cf. Table 1).

These observations suggested that the chronic administration of malachite green might damage the respiratory enzyme system, thus

creating a situation which WARBURG demanded as a basic requirement for malignancy to develop. Unlike in previous tests related to this subject, the experimenters were able to use intact higher organisms without having to rely entirely on tissue cultures or on examinations of surviving tissues or cells.

It is now four years since three groups of animals were formed for this purpose; the rats were derived from the same inbred strain and were kept under exactly the same conditions as regards tending and feeding. One of these groups was administered malachite green per os, the second one was given malachite green and at the same time a corresponding intravenous dose of cytochrome-c, while the third group was used for control purposes and was not treated at all.

Based on the assumption that a suitably critical reduction of the cytochrome-c concentration in the body or of a related component of the respiratory enzyme system could be achieved only after a number of generations, the initial plan was to administer the dye to several successive generations.

Already after the first litters produced by the test animals, it was, however, noticed that the offspring of the malachite green series exhibited distinct variability in the shape of their heads, whereas the animals of the malachite-green/cytochrome-c series, like those of the control group, were unremarkable. Moreover, one deformity occured already in the first filial generation: one animal had different eyes, namely a normal one and a micro-eye. For this reason, we decided

not to treat the animals any further so that the following findings refer exclusively to the untreated offspring of the treated parental generation.

The incidence of abnormalities increased steadily in the successive generations of the malachite green series. The animals continued to exhibit great variability in the shape of their heads, as verified by measurements; we noticed disturbances of the skeletal system with tumefaction of costal cartilage-bone boundaries, spinal anomalies, deformities of the extremities and the eyes, etc.

Lung deformities which were observed already in the second untreated filial generation were particularly noticeable. At the macroscopic level, these lungs were remarkable for their tissues which were permeated by multiple cysts, with sometimes hardly any normal lung tissue left. For this reason, we called them "grapy lungs" ("Traubenlungen"). Microscopic examinations proved that the lesions were alveolar dilations forming systems of sacculi histologically similar to those identified in human bronchiectasis.

The filial generations were likewise remarkable for the high incidence of underdeveloped forms, including well proportioned dwarves, deformed dwarves and non-viable stunted forms.

The above forms of damage in the untreated offspring occurred mainly in the second to the sixth generations, but were detected up to the tenth and twelfth generations.

Very soon, i.e. for the first time in the second filial generation, we noticed tumors which also increasingly occurred from generation to generation. First we identified lung tumors, then ovarian tumors, one kidney tumor, several generalized peritoneal carcinoses, as well as mammary neoplasms and a number of myocardial tumors. It should be noted that we never detected gastric or intestinal tumors.

The sixth and seventh filial generations showed the most remarkable incidence of tumors. The malachite-green/cytochrome-c series and the control series were not subject to any of these disturbances. They were neither affected by any kind of damage nor by tumors.

It was, of course, interesting to examine how the cytochrome-c concentration in the heart muscle changed.

The analysis of approximately 500 heart muscles taken from our animals produced the following findings (cf. Table 3):

The results clearly reveal that the malachite green series shows the lowest cytochrome-c concentration, the normal series contains medium quantities of the enzyme and the malachite-green/cytochrome-c series even shows a higher cytochrome-c concentration than the normal animals.

The amount of the cytochrome-c concentration is correlated with the incidence of tumors. The families with the highest tumor incidence likewise show the highest reduction in the myocardial cytochrome-c concentration. Vice versa, the rats with the lowest decrease in cytochrome-c were least affected by tumors.

A thorough histological examination of the tumors without exception yielded evidence of clear carcinoses which cannot be described here in detail.

The test series on approximately 5,000 rats thus reveal three separate phases:

1. The administration of malachite green causes intracellular damage which affects the cytochrome system and leads to a reduction of the cytochrome-c concentration in the cell; thus, it inevitably causes damage to the respiratory system. This damage can be prevented by simultaneously administering cytochrome-c.
2. The cytochrome-c deficiency is transmitted by heredity. Therefore, the cell damage must be linked with the hereditary factors in a so far undefined way.
3. This inherited impairment of cell respiration causes phenotypical symptoms such as deformities, stunted growth etc., i.e. patterns of embryonic damage arising from exogenic deficiency of oxygen, with which we are also familiar from studies conducted by BUECHNER and his followers.

   Furthermore, this intracellular situation stimulates the formation of malignant tumors, leading in the sixth to eighth untreated filial generation to tumor production in approximately 20 % of the animals.

   Thus these tests on intact whole animals provide experimental support to WARBURG's opinion that damage to the respiratory system

is the pre-condition of malignant degeneration. Such respiratory disturbance, however, does not lead to acute tumor formation, instead it requires a number of successive generations to arrive at a constellation of metabolic factors in the cell which causes malignant tumors to be formed.

The formation of tumors caused by the damage resulting from exposure to malachite green - this is the form of expression which we have chosen for this mechanism of malignant degeneration - may, however, be regarded as a new model for the carcinogenesis triggered by hereditary cell damage affecting, in this case, the respiratory system. This kind of tumor formation shows that carcinogens need not have a direct effect, but that malignant degeneration can be a long process requiring, as in this case, several generations to materialize.

Table 1: Cytochrome-c concentrations in the heart muscles of normal rats and of animals which were intermittently administered malachite green (ma) or malachite green and cytochrome-c (cy-c). (Cy-c determination according to the American standardization method; UMBREIT, W.W., BURRIS, R.H. and STAUFFER, J.F.: Manometric techniques. Burgess Pub. Co., Minneapolis, Minn. 1949 and 1957).

(1) Cytochrom c in mg/1 g Herzmuskel (Frischgewicht)		
(2) Normaltiere ♂	(3) Malachitgrünbehandlung ♂	(4) Malachitgrün + Cytochrom-c-Behandlung ♂
(5) Durchschnittswert von 30 Tieren 0,1400	Durchschnittswert von 30 Tieren 0,1103	Durchschnittswert von 30 Tieren 0,1865

Translation key:

1. Cytochrome-c in mg/lg of heart muscles (net weight)
2. Normal animals
3. Administration of malachite green
4. Administration of malachite green and cytochrome-c
5. Average value in 30 rats

Table 2a: Treatment of animal No. 18

(1) Datum	(2) Tägl. Dosis Ma in mg	(3) Ges.-Menge Ma in mg	(4) Würfe
29. 7. – 9. 8.	0,5	6,0	
10. 8. – 16. 8.	0,75	5,25	
17. 8. – 22. 8.	0,75	4,5	
23. 8. – 29. 8.	0,82	5,74	
(5) Kopulationstermin			
30. 8. – 10. 11.	0,79	9,48	
11. 9. – 21. 9.	0,93	11,16	
(6) Insgesamt: 22. 9.		42,13	(7) I. Wurf – 11. Junge
22. 9. – 29. 10.		36,27	
Insgesamt: 22. 10.		78,40	(8) 10 Junge leben
(9) Neuer Versuch	(10) Ma per Sonde		
24. 1. 1956	0,5		
26. 1. 1956	0,5		
28. 1. 1956	0,6		
30. 1. 1956	0,6		
1. 2. 1956	0,6		
3. 2. 1956	0,6		
5. 2. 1956	0,6		
Insgesamt: 11. 3. 1956		4,0	(11) II. Wurf – 5 Junge
11. 4. 1956			5 Junge leben
2. 5. 1956	0,5		
9. 5. 1956	0,6		
14. 5. 1956	0,6		
18. 5. 1956	0,6		
25. 5. 1956	0,6		
29. 5. 1956	0,6		
1. 6. 1956	0,6		
4. 6. 1956	0,6		
9. 6. 1956	0,6		
16. 6. 1956	0,6		
18. 6. 1956	0,6	6,5	
Insgesamt: 19. 6. 1956		6,5	(12) Tier †

Translation key:

1. Date
2. Daily dose Ma in mg
3. Total quantity Ma in mg
4. Litters
5. Day of copulation
6. Total
7. First litter - 11 young
8. 10 young still alive
9. New experiment
10. Ma per sound
11. Second litter - 5 young still alive
12. Dead animal

Table 2b: Treatment of animal No. 12

(1) Datum	(2) Tägl. Dosis in mg		(3) Ges. Menge in mg		(4) Würfel
	Ma	Cy c	Ma	Cy c	
29. 7. - 9. 8.	0,5		6,0		
30. u. 31. 7.		5		10	
1. 8. - 5. 8.		10		50	
10. 8. - 16. 8.	0,8	5	5,6		
17. 8. - 22. 8.	0,75	5	4,5		
23. 8. - 29. 8.	0,86	5	5,16		
	(5) Kopulationstermin				
30. 8. - 10. 9.	0,85	5	10,2		
11. 9. - 23. 9.	0,95	5	12,35		
6. 8. - 23. 9.		5		235	
			42,81	295	
23./24. 9.					
24. 9. - 29. 10.			32,3		(6) I. Wurf -- 4 Junge
24. 9. - 15. 11.		5		265	
			76,11	560	
24. 10.					(7) 3 Junge leben
24. 1. 1956	0,5	5			
26. 1. 1956	0,5	5			
28. 1. 1956	0,6	5			
31. 1. 1956	0,5	5			
1. 2. 1956	0,7	5			
3. 2. 1956	0,5	5			
5. 2. 1956	0,5	5			
9. 3. 1956		4			
10. 3. 1956		4			
11. 3. 1956		4			
12. 3. 1956		4			
14. 3. 1956		4			
15. 3. 1956		4			
2. 5. 1956	0,6				
10. 5. 1956	0,6	3			
16. 5. 1956	0,6	2			
19. 5. 1956	0,65	2			
22. 5. 1956	0,75	2			
23. 5. 1956		3			
24. 5. 1956			7,0	71	(8) II. Wurf -- 9 Junge
26. 5. - 25. 6.			5,6	35	
21. 6.					(9) 8 Junge leben

Translation key:

1. Date
2. Daily dose in mg
3. Total quantity in mg
4. Litters
5. Day of copulation
6. First litter - 4 young
7. 3 young still alive
8. Second litter - 9 young
9. 8 young still alive

Table 3: Cy-C concentration in the heart muscle of rats belonging to the Ma series, Ma + Cy-c series and controls. Each individual value refers to the determination of the myocardial Cy-c concentration for a group of six rats.

(1) Normaltiere (Kontrollen)	(2) Ma-Serie	(3) Ma + Cy-c-Serie
0,143	0,107	0,141
0,140	0,115	0,142
0,142	0,110	0,143
0,145	0,127	0,140
0,143	0,103	0,160
0,139	0,117	0,177
0,142	0,125	0,180
	0,110	0,178
	0,090	0,197
		0,175
(4) Mittel:		
0,142	0,112	0,163

Translation key:
1. Normal animals (control series)
2. Ma series
3. Ma + Cy-c series
4. Mean value

Plate 1

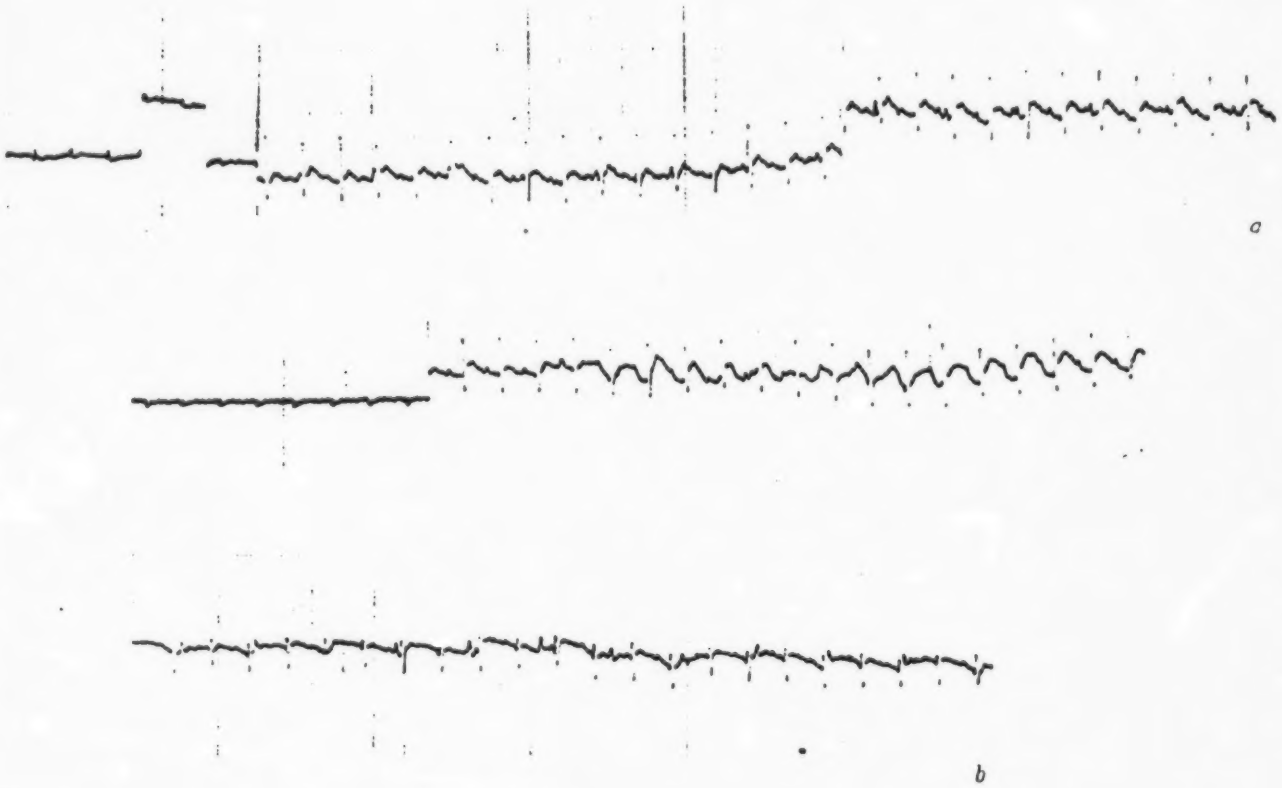

Fig. 1. ECG of a rat under the influence of different O_2/N_2 mixtures (without anesthesia)
a) ECG before the experiment, no anesthesia!
b) ECG after the administration of $12\%O_2/88\%N_2$. Elevation of T_2 which also becomes wide and awkward. Arch-shaped T_3.

Plate 2

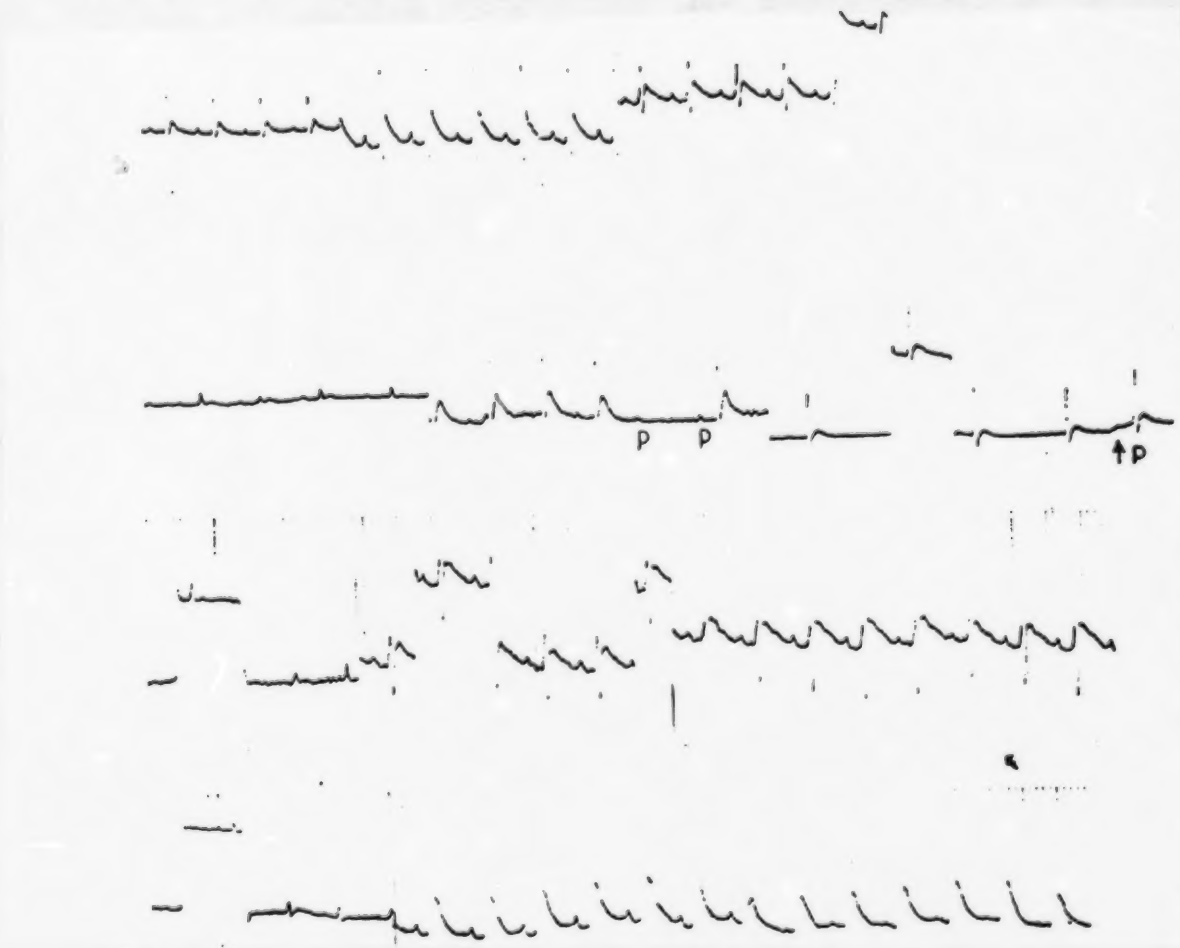

Fig. 2. ECG of a rat belonging to the offspring of animals which were administered malachite green. The offspring were not treated: various forms of pathological T waves.

Plate 3

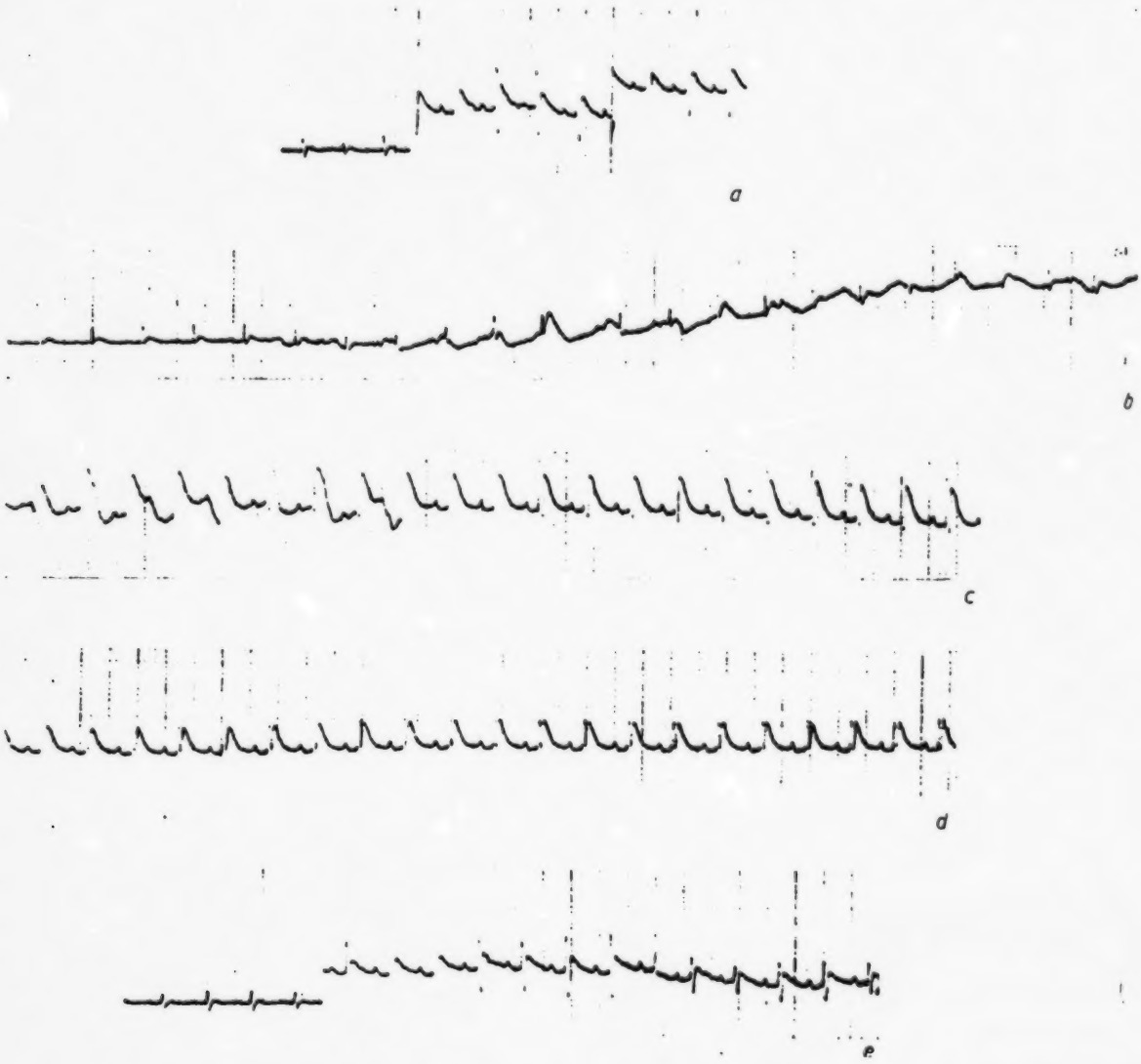

Fig. 3: Test with cytochrome-c

a) ECG before the test. Ether anesthesia.
b) ECG recorded immediately after the injection of malachite green, 3.5 mg/100 g of rat. Lead I clearly shows the increasing changes, which are evident particularly in the T wave.
c) Lead II, maximum rise in the T wave.
d) Lead III also shows tall T spikes.
e) ECG recorded 1 min. after the injection of Cytochrome-c 7mg/100 g of a rat: normalization.

Plate 4

Fig. 4. Change in the shape of the head, formation of a fox-like head in one rat

Fig. 5. Different eyes: one normal eye (right), one blind micro-eye (left)

Fig. 6. Deformity of the eyes in one rat

Fig. 7. Tumefaction of the bones in the hind legs of one rat

Fig. 8. Deformation of both forelegs: deformed toes and missing distal phalanges

Plate 5

Fig. 9. Deformations of the hind legs (toes) and the bones resulting secondarily in inflammations, due to the shedding of bone splinters, and also in gangrene of the tail

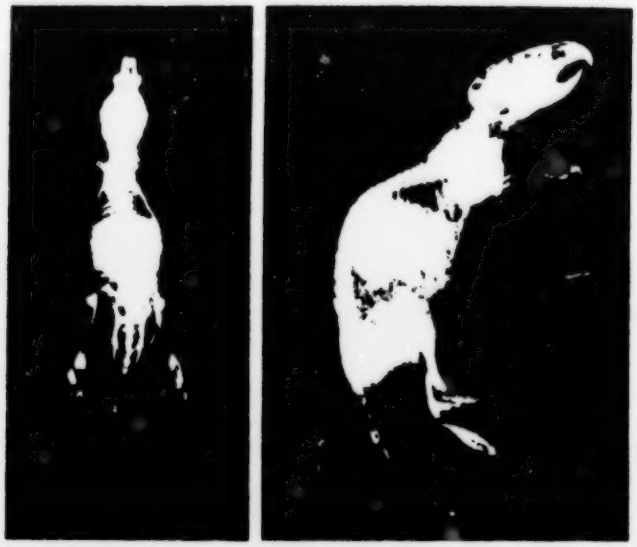

Fig. 10. Deformations of the bones in the malachite green series. Left: Lesions of the hind legs. Right: Anomalies of the coccygeal vertebrae.

Fig. 11. Stunted growth: on the right, a normal rat is shown for comparison

Fig. 12. Stunted growth: four-week-old rat weighing 12 g!

Fig. 13. Massive lung tumor in a rat

Fig. 14. Lung tumor in a rat, which has eroded the ribs on the right side

Plate 7

Fig. 15. Kidney tumor in a rat

Fig. 16. In situ ovarian tumor in a rat

Plate 8

Fig. 17. Two ovarian tumors in the same rat

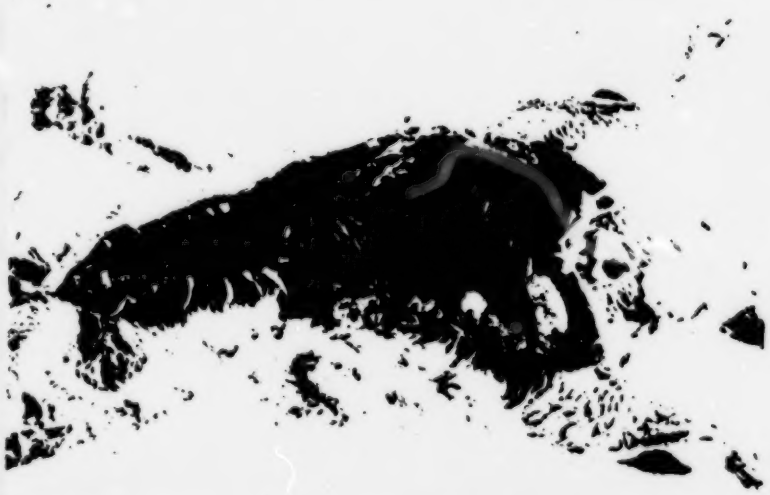

Fig. 18. Generalized peritoneal carcinosis in a rat. The entire content of the peritoneal cavity has developed into one adherent tumor.